

SMITH (T.)

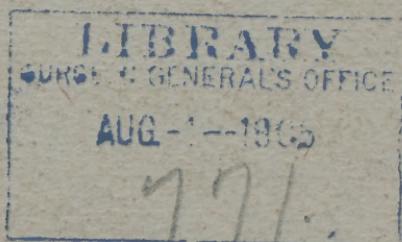
THE THERMAL DEATH-POINT OF TUBERCLE
BACILLI IN MILK AND SOME
OTHER FLUIDS

BY

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the Massachusetts State Board of Health.*)

In the comparative study of tubercle bacilli from various sources recently published,* an experiment was made to learn whether any differences towards heat would be manifested by cultures from man and from cattle. Pursuing the methods hitherto employed, I suspended the bacilli in milk and exposed the suspensions at 60° C. for different periods of time. The result was at variance with current notions of the resistance of tubercle bacilli at this temperature. A second trial gave a result quite at variance with the first, so that a third trial was made. Quite unintentionally I was drawn into a series of experiments extending over a period of more than 18 months, which have yielded several facts not anticipated, and destined to change somewhat our conceptions of the heat-resisting power of tubercle bacilli. Before detailing the tests, a brief review of the experiments of others will aid us in appreciating the necessity for the present work. In all my tests I restricted myself to the temperature of 60° C. in order to keep the work within certain narrow limits. I shall therefore confine my review to the determinations of others at that temperature.

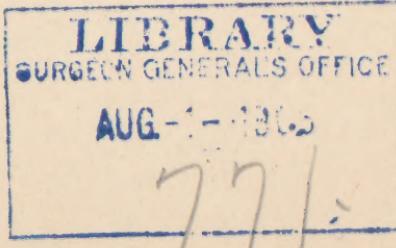
Sternberg† in 1887 inoculated a guinea-pig with sputum exposed at 60° C. for 10 minutes. The animal remained unaffected.

Yersin‡ in 1888 tested an old glycerine bouillon culture in which the

* Theobald Smith: A Comparative Study of Bovine Tubercle Bacilli and of Human Bacilli from Sputum, *JOURNAL OF EXPERIMENTAL MEDICINE*, 1898, iii, 451.

† Sternberg, Disinfection and Disinfectants, etc., by the Committee on Disinfectants, appointed by The American Public Health Association, p. 148. Concord, N. H., 1888.

‡ Yersin, *Annales de l'Institut Pasteur*, 1888, ii, 60.



bacilli appeared "sporulating." Heated for 10 minutes at a temperature of 60° C. in small, sealed tubes, they still multiplied and produced disease in rabbits.

Grancher and Ledoux-Lebard* found avian cultures dead after an exposure of 20 minutes at 60° C., but not after 10 minutes. Human cultures were dead after an exposure of 15 minutes. Bonhoff† cultivated a human tubercle bacillus of unknown history in glycerinated calf's-lung bouillon. An exposure of such a culture for 20 minutes to 60° C. destroyed the bacilli.

de Man‡ in Forster's laboratory made a series of tests with tuberculous tissue. The thermal deathpoints established by him are now generally published in text-books. He used chiefly the disintegrated, semi-fluid, cheesy matter from tuberculous udders. His exposures in sealed tubes at 60° C. may be briefly summarized. After an exposure for 15, 30 and 45 minutes 2 guinea-pigs inoculated with material from each exposure became tuberculous. A repetition of this test yielded the same results. Two guinea-pigs, inoculated with material from two separate exposures for 60 minutes, remained well. Sputum exposed for 60 minutes was equally innocuous. From these results it has been generally assumed that it takes 60 minutes at 60° C. to kill tubercle bacilli.

Woodhead§ fed milk, from a tuberculous udder, which had been exposed for 15 minutes to 60° C. to 6 guinea-pigs without any effect. Similarly, milk heated for 30 minutes had no effect on 5 guinea-pigs fed with it. On the other hand, milk heated 15 minutes at 60° C. and injected into the peritoneal cavity produced tuberculosis in 2 of 3 guinea-pigs. Of three guinea-pigs inoculated in the same way with milk heated 30 minutes one became tuberculous. In another series of experiments with milk from a tuberculous udder no disease resulted from milk heated 25 or more minutes. Milk heated for shorter periods was not injected. Experiments were also made with milk to which ground and minced tuberculous tissue had been added. In such milk artificially infected, the tubercle bacilli were in some instances still alive after two hours' exposure.

This brief survey shows that these various tests were made under such different conditions that they cannot be utilized to define the thermal deathpoint of tubercle bacilli even if the results were fairly

* Grancher and Ledoux-Lebard, *Arch. de méd. expér.*, 1892, iv, 1.

† Bonhoff, *Hygienische Rundschau*, 1892, ii, 1009.

‡ de Man, *Arch. f. Hygiene*, 1893, xviii, 133.

§ Woodhead, Report of the Royal Commission on Tuberculosis, 1895, p. 146.

concordant. There was, therefore, ample justification for a repetition of these experiments with cultures of known history.

Milk was at first used exclusively as the suspending fluid for obvious reasons. Later on it became necessary to use simpler fluids, such as bouillon, distilled water and physiological salt solution to clear up the discordant results obtained with milk. The manipulations were modified from one test to another either to remove some supposed defect of former methods or to answer certain new queries which former tests had raised. The cultures used were all isolated by me and their history is given in a former publication.* Blood serum from the dog set at 75° to 76° C. was used exclusively as the culture medium. The suspensions were made by rubbing masses of bacilli against the inner surface of sterile test-tubes near the bottom until a fairly homogeneous coating had been formed. The rubbing was done with a heavy platinum wire beaten into the form of a slender spatula. The suspending fluid was then poured into the tube and thoroughly stirred. The resulting suspension still contained many clumps of bacilli made up of a few to 30 or more rods. The tubes thus prepared were exposed in a water-bath (Experiments I, II), or else other tubes were used to which definite quantities of the original suspension had been added. In all cases the suspensions were injected directly into the peritoneal cavity of guinea-pigs in order to give the bacilli the best opportunity for multiplication. Further details are given in the following account of the tests described in the order in which they were made.

Experiment I. June 4, 1897. Bovine culture II and sputum culture II were used. Growth of former a thin layer like ground glass in appearance; growth of latter much richer. Suspensions made, as described, in tubes of moderately heavy glass having an internal diameter of 15 to 18 mm. and provided with a glass cap ground to the tube and having a narrow ventilating tube above plugged with glass wool. This kind of tube differs from ordinary test-tubes simply in having more limited ventilation. About 10 cc. of milk, previously heated to 60° C., was added with a pipette and the suspension gently stirred. The tubes were then clamped in a water-bath kept at 60° C. with occasional fluc-

* Loc. cit.

tuations of 0.5° above or below this point. From each tube about 2 cc. of milk were removed with a sterile pipette at the end of 30, 45, 60 and 75 minutes. Each time the suspension was gently stirred by forcing it back from the pipette to counteract the settling of the bacilli. Great care was taken not to soil the walls of the tube above the level of the fluid. The tubes themselves were immersed to a depth of about 10 cm., the surface of the suspension being about 5 cm. below the surface of the water-bath.

The vitality of the bacilli was tested by injecting about 1 cc. into the peritoneal cavity of guinea-pigs. Further details are given in Table I.

TABLE I (EXP. I).—SUSPENSIONS IN MILK.

Designation of culture.	Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
			Initial.	Final.		
Bovine II	30 minutes.	1	548	565	—	Chloroformed in 18 days; 6 to 8 nodules on omentum.
	45 "	1	480	514	—	Chloroformed in 39 days; several nodules on omentum.
	60 "	1	435	415	—	Chloroformed in 26 days; several nodules on omentum.
	75 "	1	375	445	—	Chloroformed in 26 days; several nodules on omentum.
Sputum II	30 "	1	448	471	—	Chloroformed in 39 days; no lesions.
	45 "	1	412	474	—	Chloroformed in 43 days; 6 nodules on omentum.
	60 "	1	415	462	—	Chloroformed in 43 days; 10 nodules on omentum.
	75 "	1	375	350	—	Chloroformed in 26 days; 6 nodules on omentum.

In this first test no inoculations were made with unheated suspensions, as it was supposed that if it takes 60 minutes to kill tubercle bacilli at 60° C. the animals receiving the milk heated 30 minutes would be equivalent to control animals. This, however, failed to be the case, as all animals remained free from disease. This unexpected result suggested the possibility that the cultures may have been dead when used. An examination of the culture records showed, however, that subcultures from the cultures used in the experiment were fertile. There was nothing in the appearance of the cultures, only 11 days old, to suggest loss of vitality.

Experiment II. June 25, 1897. Sputum culture III and swine culture I used. Both 13 days old. Growth of sputum culture rich, that

of the other less so. The procedure differed from that of Experiment I in that the milk was added cold to the smeared tube. Due allowance for the warming of the fluid is made in the general summary given in Table IX (p. 230). Coverslip preparations showed the swine-culture suspension to be denser than the other. The inoculations are given in Table II.

TABLE II (EXP. II).—SUSPENSIONS IN MILK.

Designation of culture.	Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea- pigs in grammes.		Result.	Remarks.
			Initial.	Final.		
Sputum III	0 minutes.	1	555	260	+	Dying on 27th day; chloroformed. General tuberculosis.
	15 "	1	520	545	+	Chloroformed in 46 days; lesions in omentum, lymph glands, spleen, liver.
	30 "	1	475	493	-	Chloroformed in 47 days; about a dozen nodules on omentum.
	45 "	1	487	527	+	Chloroformed in 55 days; nodules and larger necrotic foci on omentum. Tuberculous retrogastric gland; minute yellow spots in liver. Thoracic glands enlarged but free from necroses.
	0. "	1	600	..	+	Dies in 13 days. Usual acute tuberculosis following abdominal injection.
Swine I	15 "	1	550	570	+	Chloroformed in 45 days. Tuberculosis of all thoracic and abdominal glands. A few foci in spleen and liver.
	30 "	1	482	492	-	Chloroformed in 47 days. Some nodules on omentum.
	45 "	1	422	425	+	Chloroformed in 55 days. General tuberculosis.

The outcome of this experiment was quite different from that of the preceding. Only the animals which received the milk suspension heated 30 minutes may be said to have received no living virulent bacilli. Those receiving milk heated 15 and 45 minutes from both cultures were distinctly tuberculous.

Experiment III. This may be disposed of briefly as it adds nothing new to the information gained by the other tests. It is, however, of

some general interest. July 1, 1897. The udder of a cow of which one-quarter was enormously enlarged by tuberculosis and the others atrophied and giving but little milk was the starting point. The tuberculous tissue was largely broken down into a caseous, diffluent mass. From one atrophied quarter a little milk was obtained with the hope that the thermal deathpoint of tubercle bacilli naturally suspended in that fluid might be obtained as a check upon the artificial suspensions. Though tubercle bacilli were not found in it with the microscope still the inoculations were made; 2 cc. of milk heated 0, 20, 35, 50 and 65 minutes were injected, each lot into 2 guinea-pigs. Killed from 6 to 9 weeks after inoculation, they were found free from disease. The milk was probably free from tubercle bacilli and the disease strictly limited to one-quarter.

Experiment IV. This test with bovine caseous material suspended in ordinary peptone bouillon was made mainly to determine whether the tubercle bacillus might exist in some more heat-resisting stage in caseous masses in which multiplication seems to be at a standstill.

TABLE III (EXP. IV).—BOUILLON SUSPENSIONS OF CASEOUS MATTER (CATTLE).

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	0.6	342	404	+	Chloroformed in 42 days. General tuberculosis.
0 "	2.	482	484	+	Chloroformed in 27 days. General tuberculosis.
0 "	2.	657	640	+	Chloroformed in 39 days. General tuberculosis.
20 "	"	646	693	-	Chloroformed in 31 days. One nodule on omentum.
" "	"	383	505*	-	Chloroformed in 55 days. No lesions.
35 "	"	445	622	-	Chloroformed in 54 days. No lesions.
" "	"	612	701	-	Chloroformed in 31 days. No lesions.
50 "	"	485	593†	-	Chloroformed in 106 days. No lesions.
" "	"	339	445	-	Chloroformed in 31 days. One nodule on omentum.
65 "	"	567	592†	-	Chloroformed in 129 days. One nodule on omentum.
" "	"	329	418	-	Chloroformed in 18 days. No lesions.

* Pregnant when chloroformed.

† Had given birth to a litter of healthy young 4-6 weeks before it was chloroformed.

Nov. 26, 1897. Completely caseous matter from the lungs of a cow was scraped into a small dish and kept on ice until next day. It was

ground in a sterile mortar with bouillon and the turbid fluid passed through several thicknesses of linen. In the densely turbid, pale yellowish fluid, consisting microscopically of fatty debris chiefly, tubercle bacilli were not found. 7 cc. were carefully transferred with a pipette to a number of test-tubes with ground glass cap. These were immersed in the 60° bath so that the surface of the fluid was 5 cm. below the level of the bath. A tube was removed at the end of 20, 35, 50 and 65 minutes. Table III (p. 222) shows that even 20 minutes was sufficient to destroy the bacilli, and that in caseous tissue tubercle bacilli do not possess any greater power of resistance to heat than in cultures on serum.

TABLE IV (EXP. V).—A. SUSPENSION OF BOVINE BACILLI IN BOUILLON.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	0.5	581	468	+	Dies in 17 days. Acute tuberculosis.
20 "	1.5	544	582	-	Chloroformed in 52 days; a dozen nodules on omentum.
(Bacilli from nodules of preceding case.)		340	405	-	Chloroformed in 37 days; no lesions.
35 minutes.	1.5	554	638	-	Chloroformed in 43 days; a dozen nodules on omentum, slight hyperplasia of lymph glands.
50 "	"	528	607	-	Chloroformed in 49 days; six nodules on omentum.
65 "	"	349	498	-	Chloroformed in 58 days; nodules on omentum, one in ovarian ligament.

B. SUSPENSIONS IN MILK.

(Milk not infected.)	3.5	421	..	-	Dies in 7 days of streptococcus infection; abscesses in omentum and between spleen and liver.
(Milk not infected.)	"	401	..	-	Dies in 7 days of streptococcus infection.
0 minutes.	1.5	591	430	+	Dies in 15 days; acute tuberculosis.
20 "	"	401	478	+	Chloroformed in 52 days. General tuberculosis.
35 "	"	510	671	+	Chloroformed in 43 days. Tuberculosis of lymph glands; slight infection of liver and spleen.
50 "	"	401	514	+	Chloroformed in 49 days. General tuberculosis.
65 "	"	369	510	+	Chloroformed in 58 days. General tuberculosis.

Experiment V. The preceding tests taken together suggested that tubercle bacilli may be much more resistant to heat in milk than in

bouillon. This suggestion was tested in the following manner with bovine culture III:

A. Bouillon series, Feb. 15, 1898. The culture, 17 days old, was suspended in bouillon having a reaction equivalent to 1.45 per cent. of a normal acid solution (phenolphthalein). Ordinary, cotton-plugged thin-walled test-tubes were used, 15 to 18 mm. in diameter and 15 cm. long. Each tube received 2 cc. of the suspension and was immersed to a depth of 10 cm. in the bath. One was removed at the end of 20, 35, 50 and 65 minutes.

B. Milk series. The tubes were prepared in the same way. The milk was about 6 hours old, kept in the refrigerator. Acidity 1.9 per cent.

The result of the inoculations given in Table IV (p. 223) shows that the bacilli in the bouillon suspensions had been killed, while some of those in the various milk tubes had survived.

TABLE V (EXP. VI).—A. SUSPENSION OF BOVINE BACILLI IN WATER.

Time of exposure at 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
15 minutes.	1.5	304	548	—	Killed in 64 days. Some nodules on omentum.
25 "	"	358	416	—	Killed in 35 days. Some nodules on omentum.

B. SUSPENSION IN MILK.

(Milk not infected.)	3.5	732	..		Dies in 6 days. Abscesses in abdominal cavity. Streptococcus infection.
(Milk not infected.)	"	379	350	—	Chloroformed in 31 days. Large abscess in abdominal cavity due to minute bacilli.
(Milk not infected.)	"	444	591	—	Chloroformed in 59 days. No lesions. Milk from same dairy but from a lot 11 days later than preceding.
0 minutes.	1.	342	..	+	Dies in 9 days. Acute tuberculosis and streptococcus infection.
20 "	1.4	317	360	—	Killed in 61 days. Several nodules on omentum.
35 "	2.	409	411	+	Killed in 29 days. Tuberculosis of omentum, peritoneum and lymph glands.
50 "	1.5	363	453	—	Killed in 64 days. Several nodules on omentum.
65 "	2	370	508	+	Killed in 64 days. Slight tuberculosis of lymph glands of abdomen, liver and spleen.
80 "	2	420	595	—	Killed in 64 days. A few nodules on omentum, 2 minute ones on testicles.

Experiment VI. April 28, 1898. This is an exact counterpart of Experiment V with certain minor modifications. The culture is a young (seven-day) culture of bovine bacillus IV. The suspensions were made, as before, in milk, and in distilled water. They were exposed in cotton-plugged test-tubes. The ready destruction of the bacilli in water, their irregularly persistent vitality in the milk is again demonstrated in Table V (p. 224). Cultures from the water suspensions confirmed the inoculation results. The control unheated suspension gave rise to a vigorous culture on dog's serum, the cultures from the heated suspensions remained sterile.*

TABLE VI (EXP. VII).—A. SUSPENSION OF BOVINE BACILLI IN WATER.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	+	Culture positive.
12 "	1.5	406	474	-	Culture negative. Animal killed in 75 days. Slight hyperplasia of mesenteric and sternal glands. Several nodules on omentum and one on abdominal wall.
23 "	1.5	366	490	-	Culture negative. Animal killed in 56 days. Some nodules on omentum.

B. SUSPENSION IN MILK.

(Milk not infected.)	2.0	384	664	-	Chloroformed in 75 days. No lesions.
0 minutes.	0.5	375	270	+	Dies in 32 days. General tuberculosis. Abscess in omentum containing cocc.
17 "	1.5	367	448	-	Killed in 56 days. Some nodules on omentum.
32 "	"	353	443	-	Killed in 56 days. Some nodules on omentum.
47 "	"	403	451	-	Killed in 56 days. Some nodules on omentum.
62 "	"	385	508	-	Killed in 56 days. Some nodules on omentum, and cheesy focus in subcutis at point of injection.
77 "	"	395	498	-	Killed in 56 days. Some nodules on omentum.

Experiment VII. May 18, 1898. The procedure differed from that of former tests in that the suspensions were exposed in com-

* Cultures from milk suspensions were not attempted because of the anticipated presence of contaminating bacteria. To sterilize the milk before use by discontinuous boiling would obviously fail to imitate natural conditions.

pletely submerged, sealed glass tubes. An old culture was used to determine if more resistant bacilli appear after a time.

Bovine IV, grown for 30 days on the same lot of dog's serum on which the young culture of the preceding experiment had grown was used. The milk had an acidity of 1.7 per cent. Parallel exposures in distilled water were also made. The tubes were made of ordinary glass tubing having an internal diameter of 6 mm. Both ends were drawn out in the flame. One end was sealed at once. About 2 cc. of the suspension was inserted through the other opening with a delicate pipette and the latter then sealed in the flame. This proved a better method than drawing the suspension up first and then sealing as it prevented the usual sputtering when the sealing is attempted and the indiscriminate distribution of tubercle bacilli. Each sealed tube contained one-half to one-third its volume of air. They were laid horizontally on a rack about 7 cm. below the surface of the bath close to the thermometer bulb. The results of the inoculation are given in Table VI (p. 225).

The outcome of this test was a surprise, for not one of the guinea-pigs inoculated with heated suspensions became tuberculous. For the water suspensions the culture test agreed with the animal test. A vigorous culture was obtained from the unheated suspension in two weeks. The other tubes were sterile after 4 weeks. It will be at once thought that the use of the sealed tubes accounts for this, but why should the suspensions in fluids other than milk give uniform results in test-tubes? The remaining two experiments, I think, give a satisfactory answer to this query.

Experiment VIII. August 12, 1898. In this test bovine culture VI was used. The bacilli were exposed in sealed tubes. The milk, kept over night in the refrigerator, had a heavy layer of cream on it next morning. It was thoroughly mixed before use. Acidity 1.5 per cent. Suspensions in normal salt solution were also made. The result of the test given below in Table VII did not differ materially from that of the preceding test. In the 12-minute tube the bacilli suspended in salt solution were not all destroyed. The guinea-pig inoculated with the milk suspension exposed for 17 minutes had a suspicious subcutaneous lymph gland with which unfortunately fresh inoculations were not made, so that this case remains in doubt. However, the lesion was so slight, if due to living bacilli, that one bacillus may have caused it.

TABLE VII (EXP. VIII).

A. SUSPENSION OF BOVINE BACILLI IN NORMAL SALT SOLUTION.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
12 minutes.	1.5	338	464	+	Chloroformed in 106 days. Tuberculosis of both testicles, of left popliteal, and of lumbar gland. All foci softened. Bronchial glands tuberculous; still firm.
22 "	"	376	508	-	Chloroformed in 106 days. Two small, softened nodules on omentum; one on testicle.

B. SUSPENSION IN MILK.

(Milk not infected.)	3.5	251	640	-	After 105 days weighs 640. Alive after 5½ months.
0 minutes.	0.5	373	..	+	Dies in 9 days of acute tuberculosis.
17 "	1.5	388	494	+	Killed in 57 days. Slightly enlarged and partly necrotic popliteal gland.
32 "	"	398	532	-	Killed in 57 days. Several 2-3 mm. nodules on omentum.
47 "	"	366	545	-	Killed in 68 days. No lesions.
62 "	"	298	423	-	Killed in 63 days. Several nodules on omentum.
77 "	"	267	479	-	Killed in 63 days. Slightly conspicuous retrogastric and thoracic lymph glands.

Experiment IX. Nov. 17, 1898. In this test bovine culture III, used in Experiment V, was again employed. The suspensions were prepared from a vigorous culture 13 days old. Its vitality was demonstrated by the rapid, rich growth of a subculture made at the same time with the suspensions. The bacilli suspended in distilled water were exposed in the test-tubes with ground glass cap, each tube receiving 2 cc. The milk suspensions were exposed both in these tubes (1 cc.) and in sealed tubes. The outcome of the tests made thus far had suggested the theory that the occasional survival of bacilli leading to the irregular results tabulated might be due to the formation of the surface pellicle into which the bacilli are carried by particles of fat. Here their thermal deathpoint might be higher than when submerged in a watery fluid. Hence the pellicle which had formed during the exposure in the test-tubes was in part removed with a platinum loop and stirred in with the rest of the milk to be injected. The milk used had a layer of cream (formed over night) equivalent to 14 per cent. of the whole column. Acidity 1.67 per cent. The bore of the sealed tubes was 4 mm. They contained about 1.5 cc. milk and some air. Coverslip preparations dem-

onstrated the presence of large numbers of bacilli chiefly in clumps. The results are summarized in Table VIII.

TABLE VIII (EXP. IX).—A. SUSPENSION OF BOVINE BACILLI IN DISTILLED WATER EXPOSED IN CULTURE TUBES WITH GLASS CAP.

Time of exposure to 60° C.	Quantity injected in cc.	Weight of guinea-pigs in grammes.		Result.	Remarks.
		Initial.	Final.		
0 minutes.	0.5	384	..	+	Dies in 25 days of tuberculosis. Suspension made from the same culture 12 days after the general experiment to replace milk controls (see below).
10 "	1.5	382	418	+	Killed in 62 days. Tuberculosis of lymph nodes, liver, spleen, peritoneum.
15 "	1.5	372	438	+	Killed in 61 days. Tuberculosis of lymph nodes chiefly.
20 "	1.5	302	331	+	Killed in 61 days. Generalized tuberculosis.

B. SUSPENSIONS IN MILK IN SEALED PIPETTES.

(Milk not infected.)	4.0	524	..		Dies in 48 hours of streptococcus peritonitis and septicæmia.
0 minutes (control).	0.5	537	..	+	Dies in 6 days of streptococcus infection and tuberculosis (?).
7 minutes.	1.5	450	500	+	Killed in 62 days. Tuberculosis of lymph nodes. Slight infection of liver and spleen.
12 "	1.5	441	503	+	Killed in 62 days. Slight disease of lymph nodes. Tuberculosis of left scrotal sac.
17 "	0.5	491	605	-	Killed in 62 days. Slight localized thickening of omentum.
32 "	1.5	354	..	-	Dies in 15 days.* Six or seven small nodules on omentum; 2 on mesocolon.
47 "	1.5	368	492	-	Killed in 62 days. Several softened nodules on omentum.
62 "	1.5	303	330	-	Killed in 62 days. Some local thickenings of omentum.

C. SUSPENSIONS IN MILK IN CULTURE TUBES AS IN A.

20 minutes.	1.5	378	275	+	Dies in 38 days. General tuberculosis.
35 "	1.5	272	291†	+	Dies in 52 days. General tuberculosis.

* Diphtheritic paralysis. Had been used for antitoxin test 24 days before inoculation. Only a slight transient swelling followed.

† Weight 10 days before death.

This test strengthens the supposition that the pellicle which forms on milk in test-tubes during heating is responsible for the increased resistance of milk suspensions of tubercle bacilli and the irregular

results hitherto obtained.* The two guinea-pigs inoculated with milk exposed 15† and 30† minutes together with some of the pellicle died of tuberculosis in 38 and 52 days respectively, while two other guinea-pigs inoculated with suspensions in milk and water exposed only 5† minutes were found with but slight lesions after 60 days.

A summary of all the tests made is given in Table IX (p. 230), together with some additional data concerning the cultures used. The figures in parentheses following the figures giving the total exposure at 60° C. are an approximate correction representing the actual time of exposure at that temperature.

A brief explanation of the tabulated autopsy notes of the guinea-pigs seems necessary in view of the fact that the conclusions to be drawn rest chiefly on them. It will be observed from the tables that in many cases small nodules were found on the omentum without any traces of tuberculosis in other organs, even the nearest lymph nodes. These nodules were studied histologically at different stages, although it was manifest from the purely local character of the lesions and the knowledge already gained from the researches of Prudden and Hodenpyle and of Straus concerning the action of dead tubercle bacilli that these nodules were the product of dead bacilli swept together into a mass in some way on different places on the omentum. It would be beyond the scope of this article to go into minute details concerning the structure of these nodules. Suffice it to say that within two to four weeks after the intra-peritoneal injection they are one to three mm. in diameter and translucent in appearance. They consist, then, of epithelioid and lymphoid cells, among which are many nuclear figures. In this stage the mass may contain roundish cell groups closely resembling the "germinative centres" of follicles in lymph nodes. In later stages the larger nodules undergo softening centrally, and the creamy-white mass there formed contains readily

* This pellicle, which is familiar to all who have scalded milk, is a feebly cohesive mass easily washed out in patches for microscopic examination by diluting the heated milk in water. The patches consist of fat globules and an amorphous, cohesive substance of slight refrangibility, by which they are evidently held together.

† These figures are corrected by subtracting the time required to bring the fluid to 59.5° C.

TABLE IX.—SUMMARY OF EXPERIMENTS.

Source of bacilli.	Total period of cultivation on dog's serum.	Age of culture used.	Nature of suspending fluid.	Tubes used.	Result. (The figures signify minutes, the sign + tuberculosis, the sign - no tuberculosis.)
Caseous material from cow's lung.	9 mos. 27 days	17 days	Peptone bouillon	Culture tubes with glass cap	-20(15), -35(30), -50(45), -65(60)
Bovine culture III	11 mos. 26 days	7 days	Peptone bouillon	Test-tubes with cotton plug	-20(15), -35(30), -50(45), -65(60)
Bovine culture IV	13 mos. 30 days	Distilled water	Test-tubes with cotton plug	-15(10), -25(20)	
Bovine culture IV	7 mos. 19 days	Distilled water	Sealed glass tubes	-12(10), -22(20)	
Bovine culture VI	18 mos. 29 days	Normal salt sol.	Sealed glass tubes	+12(10), -22(20)	
Bovine culture III	6 mos. 7 days	Distilled water	Culture tubes with glass cap	+10(5), +15(10), +20(15)	
Bovine culture II	6 mos. 14 days	Milk	Culture tubes with glass cap	-30, -45, -60, -75	
Sputum culture II	4 mos. 10 days	Milk	Culture tubes with glass cap	-30, -45, -60, -75	
Sputum culture III	13 mos. 17 days	Milk	Culture tubes with glass cap	+15(10), -30(25), +45(40)	
Swine culture I	9 mos. 27 days	Milk	Culture tubes with glass cap	+20(15), +35(30), +50(45), +65(60)	
Bovine culture III	11 mos. 26 days	17 days	Test-tubes with cotton plug	-20(15), +35(30), -50(45), +65(60)	
Bovine culture IV	13 mos. 26 days	7 days	Test-tubes with cotton plug	-17(15), -32(30), -47(45), -62(60), -77(75)	
Bovine culture IV	7 mos. 19 days	30 days	Sealed glass tubes	+17(15), -32(30), -47(45), -62(60), -77(75)	
Bovine culture VI	18 mos. 29 days	10 days	Sealed glass tubes	+15, +12(10), -17(15), -32(30), -47(45), -62(60)	
Bovine culture III	{ 18 mos. 29 days	13 days	Culture tubes with glass cap	+20(15), +35(30)	

demonstrable tubercle bacilli. The softening is essentially a process of caseation, never of suppuration. The necrosis is sharply demarcated from the enveloping dense outer layer of the nodule and thereby differs clearly from the progressive peripheral invasion of living bacilli. It is not possible, however, to make trenchant distinctions between early lesions due to living and to dead bacilli, and so far the progressive metastatic nature of the disease as recognized with the unaided eye seems to me the most satisfactory guide, provided a sufficient period of time (at least 50 days) be allowed to elapse between the inoculation and the examination of the animal. In but one instance (Experiment V) the bacilli from a softened focus due to dead bacilli were injected into a guinea-pig. The result was negative.

There is nothing of importance to be recorded concerning the macroscopic characters of the inoculation disease in guinea-pigs. Its protean character, as exemplified by the diverse localizations, makes a very thorough examination of every animal essential. In general, the retarded disease has its chief seat in the lymph nodes, which may attain a large size. This is particularly true of the retrogastric and lumbar, and of the bronchial and neighboring sternal nodes, whose large dimensions with visibly intact lungs suggest the bovine type of disease. In several cases the testicles and surrounding scrotal tissue were involved precisely as in glanders (following either subcutaneous or intro-abdominal inoculation). It is probable that in these experiments male guinea-pigs would be preferable, since the disease may be recognizable during life. In no case was there any evidence of air infection. In fact, I have not encountered it at any time among the animals kept for several years in the room used for these experimental animals. It is again evident from these tables that the weight of the guinea-pig is no guide in determining the presence or absence of tubercular foci.

The foregoing experiments demonstrate that tubercle bacilli are no more resistant to heat than many other bacilli not producing spores, and that at 60° C. destruction is complete in 15 to 20 minutes. Even after exposures lasting 10 minutes the bacilli were dead in most

instances. After 5-minute exposures the inoculation disease produced in guinea-pigs was greatly retarded, even though three times the control dose was injected.* When, however, milk is used as the suspending fluid, the formation of a surface pellicle into which bacilli are carried by fat globules shields them from the effect of the heat so that they may survive an exposure of 65 minutes. The peculiarly irregular results obtained by the Royal Commission† are probably to be explained in the same way. The importance of a clear understanding of this phenomenon in the pasteurization of milk is obvious, and it remains to be seen how far bottled milk may be freed from tubercle bacilli without resorting to the higher temperature of 68° C. now generally employed. Probably a complete immersion, or else a complete filling of the receptacle, may furnish the conditions desired.

These conclusions may be objected to on the ground that the tubercle bacilli may have suffered in power of resistance during cultivation, but the experiment with caseous material from cattle (IV) will, I think, silence this objection. The different results obtained by de Man, already quoted and now generally accepted, are probably due to the fact that he used caseous material or else tuberculous tissue, which he ground up in a mortar and only diluted with salt solution "when necessary." Such dense suspensions cannot be used to determine the thermal deathpoint to be compared with those of other bacilli, nor can they be regarded as imitating the conditions under which tubercle bacilli appear in milk.

The method of using narrow sealed tubes has shown itself superior to that which employs ordinary test-tubes so far as milk suspensions are concerned. When other fluids were used the results were unequivocal and in harmony with the other method, excepting, per-

* It should be borne in mind that the suspensions used were quite dense. In a small drop spread out on cover glasses, dried and stained, large numbers of bacilli singly and in clumps were seen in every field of a $\frac{1}{12}$ objective.

† Woodhead, *loc. cit.*

haps, in the last experiment, in which an increased resistance of the tubercle bacilli in water is manifest.*

It simply remains to point out the possibility that the resistance of other pathogenic bacteria to heat may be increased in milk. Hitherto all practical details in sterilization have been based on the thermal deathpoint of bacteria in bouillon and other fluids.

CONCLUSIONS.

1. Tubercle bacilli when suspended in distilled water, normal salt solution, bouillon and milk, are destroyed at 60° C. in 15 to 20 minutes. The larger number are destroyed in 5 to 10 minutes.
2. When tubercle bacilli are suspended in milk, the pellicle which forms during the exposure at 60° C. may contain living bacilli after 60 minutes.

* This test was repeated, after the completion of this article, for another purpose with the same culture and under the same conditions, and the same resistance to a net exposure of 15 minutes was noted. In the suspensions in water delicate floating fragments of a pellicle of bacilli were noticed which could not be submerged by vigorous shaking. Pellicles behaving similarly are frequently seen on bouillon cultures of colon and typhoid bacilli. That this phenomenon was related to the slightly increased resistance of the culture seems probable in view of the results obtained with milk suspensions in sealed pipettes. In any case those who are inclined to repeat these tests would do well to immerse completely all suspensions whether in milk or water so as to expose such floating bacilli to the same conditions of heat and moisture under which the suspended bacilli are.

